LIFE HISTORIES OF *SCYMNUS BIPUNCTATUS* KUGELANN (COLEOPTERA: COCCINELLIDAE) AND *CHRYSOPA* SP. (NEUROPTERA: CHRYSOPIDAE): POTENTIAL AUGMENTATIVE BIOCONTROL AGENTS FOR THE MEALYBUG *DYSMICOCCUS BREVIPES* (COCKERELL) (HEMIPTERA: PSEUDOCOCCIDAE) IN VIETNAM

VU THI NGA[1], ROD EASTWOOD[2], NGUYEN THI CHAT[1] and PHAM VAN LAM[3]

[1]*Nong Lam University, Ho Chi Minh City, Vietnam*

[2]*Australian School of Environmental Studies, Griffith University, Nathan, Qld 4111*

[3]*National Institute of Plant Protection, Ha Noi, Vietnam*

Abstract

Mealybugs are serious pests of crops in Vietnam but there are several indigenous natural enemies that may be useful for their control. Life history data and the results of breeding trials are presented on two indigenous predator species, *Scymnus bipunctatus* Kugelann and *Chrysopa* sp. The suitability of both species for mass rearing and for release as augmentative biological control agents are assessed. Both predator species responded well under laboratory conditions and readily attacked the target species *Dysmicoccus brevipes* (Cockerell). Their high levels of fecundity, short generation times and high survivability in captivity suggest they might be successful when reared in adequate quantities for release in augmentative biological control programmes.

Introduction

One of the most serious pests of crops in Vietnam is the mealybug *Dysmicoccus brevipes* (Cockerell) (Waterhouse 1993a). It causes heavy damage in pineapple, coffee, mango, papaya, guava, banana, soursop and custard apple plantations, and imposes a financial burden on many communities. Mealybug infestations are difficult to eradicate because crawlers (larvae) wedge themselves in plant roots, crotches, slots of fruit and leaf folds where pesticides cannot reach them. Furthermore, side effects from the largely uncontrolled use of pesticides (*e.g.* Paul and Hai 1999) have resulted in contamination of the water table in some areas of Vietnam and excessive pesticide residues on food (Bien *et al.* 2000). Thus, the identification and utilisation of augmentative biological control agents for crop insect pests in Vietnam has become a priority (Cam 1995).

Fortunately, mealybug infestations have been successfully controlled in many countries using a variety of predators and parasites. For example, the ladybug *Cryptolaemus montrouzieri* Mulsant and a parasitic wasp, *Coccidoxenoides peregrinus* (Timberlake), were used to control the mealybug *Planococcus citri* (Risso) in Australia (Waterhouse and Sands 2001). *C. montrouzieri* was also introduced, together with the parasitic wasp *Anagyrus kamali* Moursi, to control the mealybug *Maconellicoccus hirsutus* (Green) in the Caribbean (Goolsby *et al.* 2002).

Mealybugs are so called because of the white waxy covering on their bodies that gives the impression they were rolled in flour. The wax helps protect them from excessive heat and moisture loss, and from contamination with their own excreta (Gullan and Cranston 2005). They secrete sticky honeydew that is attractive to nectar-seeking insects such as ants; however, excess honeydew adheres to leaf surfaces and attracts mould growth. Excessive mould growth is unsightly on the fruit and difficult to remove, and it can inhibit photosynthesis, thus weakening the plant (Elmer and Brawner 1975, McGavin 1993). Mealybugs feed on plant sap through a long, strawlike mouthpart, or stylet, after inserting it into the plant tissue. Damage to a plant is also caused by depletion of sap causing yellowing or loss of foliage and poor fruit set.

Classical biological control involves the importation and release of exotic control species. However, the use of endemic natural enemies where they exist is preferable because, for example, there is less likelihood of any impact on non-target species (Hoddle 2004). Augmentation and conservation of natural enemies is preferred and is an important first step in developing an integrated pest management scheme (Cam 1995). This paper documents life history data for two species of mealybug predators identified in Vietnam, namely the 'two-spotted ladybug' beetle *Scymnus bipunctatus* Kugelann and a green lacewing, *Chrysopa* sp. Both were reared in the laboratory at Nong Lam University, Ho Chi Minh City, as a preliminary study to test their suitability for mass rearing and for release as augmentative biocontrol agents for mealybugs in Vietnam.

Materials and methods

Fieldwork was undertaken by one of us (VTN) in Binh Chanh District, Ho Chi Minh City (10°46'N, 106°43'E), between August 2001 and March 2005, to locate and identify potential indigenous biological control agents for mealybug pests. Two mealybug predators, *Scymnus bipunctatus* and a *Chrysopa* sp., were reared successfully in breeding trials detailed below. Voucher specimens of *S. bipunctatus* (VTN-01 - larvae, VTN-02 - adults) and the *Chrysopa* sp. (VTN-03 - larvae, VTN-04 - adults) are lodged in the Research and Technology Transfer Centre, Nong Lam University, Vietnam, and additional specimens are lodged at La Trobe University in Victoria, Australia. Ants, including a small black species, were observed attending *D. brevipes* in the field but these have not been identified.

S. bipunctatus and the *Chrysopa* sp. were bred in the laboratory of Nong Lam University, Thu Duc District, Ho Chi Minh City, Vietnam, from August 2003 to September 2004 (*S. bipunctatus*), and from January to April 2004 (*Chrysopa* sp.). Large numbers of *D. brevipes* were reared on immature bananas in clear plastic breeding boxes (10.5 cm long x 7.5 cm wide x 4.5 cm high) with fine netting glued across a hole in the lid. Larvae of *S. bipunctatus* and *Chrysopa* sp. were reared separately on *D. brevipes* in these containers.

Slightly larger containers were used when pairing adults of *S. bipunctatus* and *Chrysopa* sp. for breeding and, in the boxes for breeding *Chrysopa* sp., some cotton-balls soaked in honey were provided as food for the adults. All food was changed daily except in the box containing adult *S. bipunctatus*, which was changed every five days. All experiments were conducted under ambient temperature and relative humidity.

In order to gauge the range of variability in life history and feeding parameters, four sets of experiments were conducted, each using 25 individuals (predators). Observations on developmental stages and the counting of eggs were conducted daily (*S. bipunctatus* eggs were counted every five days). Several parameters of the predator species were measured, including longevity, feeding rates, fecundity, survival rates and physical morphology. Survival rates for all predators were estimated by determining the proportion of adults that emerged from their pupae after completing their life cycles in captivity. The predacious effects of final instar larvae of *Chrysopa* sp. and *S. bipunctatus* on mortality of adult *D. brevipes* were calculated and corrected to the control mortality using Abbott's formula ($PT = (1 - T_A/C_A) \times 100$), where T_A is treatment survival and C_A is control survival (Abbott 1925). For this experiment, mealybugs were fed on China squash suspended in a net house 2.1 m x 1.5 m x 1.8 m high; there were three replicates of 10 *Chrysopa* sp. or 25 *S. bipunctatus* for each treatment. Daily temperature and relative humidity (RH) readings were taken, each record being the mean of three readings taken at 0630, 1130 and 1730 hrs. Temperature and RH per batch was the average taken from all breeding days. Morphological measurements and fecundity were measured on 30 individuals at an average temperature of 28.6 °C, RH 80.2% (April to September 2004).

Results

Field observations

Dysmicoccus brevipes was the most commonly encountered mealybug in the field. However, other pseudococcid pest species, such as *Ferrisia virgata* (Cockerell), *Planococcus lilacinus* (Cockerell), *P. citri*, *Maconellicoccus hirsutus* and *Rastrococcus spinosus* (Robinson), were also found. Several predator or parasitic species from at least four different orders were found attacking the mealybugs. These included *Eublemma amabilis* Moore (Lepidoptera: Noctuidae), *Spalgis epius* Westwood (Lepidoptera: Lycaenidae) and *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae), in addition to the *Chrysopa* sp. and *S. bipunctatus* investigated in this study. Breeding trials were also undertaken with *E. amabilis* (with limited success) but the predacious butterfly *S. epius* could not be bred successfully. Additional trials were conducted to test the predators' feeding rates on *Ferrisia virgata* and *Planococcus lilacinus* but the results are not reported here. Several thousand specimens of *S. bipunctatus* and *Chrysopa* sp. were successfully reared during the course of this study.

Biology of Scymnus bipunctatus

In the wild, *S. bipunctatus* was commonly found attacking mealybugs, including *Ferrisia virgata, Maconellicoccus hirsutus, Planococcus lilacinus* and *P. citri*, in addition to *Dysmicoccus brevipes*. Lifespan of juvenile *S. bipunctatus* averaged between 26.9 and 30.1 days, depending on the season. During October-December (av. 27.5 °C and RH 82.7%), the life cycle appeared to be longer than during February-April (av. 28.3 °C and RH 69.3%), although the biggest increases occurred during the egg and pupal stages (Fig. 1). Egg and pupal stages of the life cycle were the longest, while duration of the second instar larva was the shortest (Fig. 1). *S. bipunctatus* moulted three times. Fourth instar larvae consumed an average of 2.1 *D. brevipes* adults per day (range 1-3).

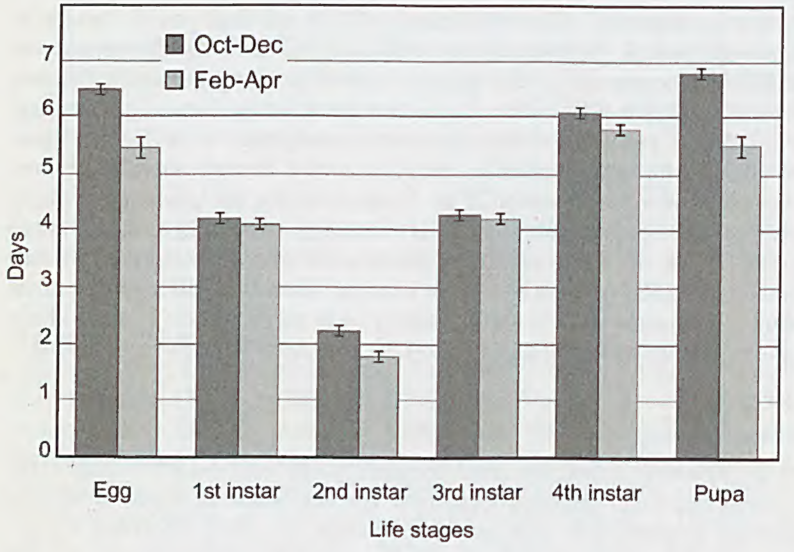

Fig. 1. Seasonal variation in the development times of *S. bipunctatus* early stages. Standard error bars shown.

Adult *S. bipunctatus* averaged 1.8 mm long (SE = 0.1) and 1.2 mm wide (SE = 0.1). When feeding on mealybugs, adult *S. bipunctatus* preferred to attack small larvae rather than large ones and they consumed an average of 63.4 (SE = 4.6, range 20-97) first instar *D. brevipes* larvae per day, or 1.6 final instar larvae per day. *S. bipunctatus* adult lifespan ranged from 30-163 days, with an average of 77.1 days (Table 1). Thus, under good conditions, each adult *S. bipunctatus* could eat nearly 5000 small *D. brevipes* larvae during its lifetime.

Once the small larvae were consumed, *S. bipunctatus* attacked mealybugs of any size. In the feeding trial, corrected prey mortality exceeded 95% by the third day (Table 2). Female *S. bipunctatus* laid an average of 222.3 eggs (SE = 11) at an average temperature of 28.6 °C and RH 80.2%.

Mean adult longevity increased during the course of the study and this was inversely related to humidity (Table 1); however, a correlation analysis found that the trend was non-significant (r = -0.724, P = 0.27), most likely due to the small number of datum points (n = 4). Overall survival rate of *S. bipunctatus* during the course of the study was 82.5%.

Table 1. Adult *Scymnus bipunctatus* longevity.

Batch	Temperature (°C)		Relative humidity (%)		Experiment duration	Adult stage (days)	
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range		$\bar{x}$ ± SD	Range
1	28.0 ± 0.2	24.5-31.3	84.2 ± 0.9	74.5-90.7	19/8/03-22/11/03	63.1 ± 9.5	30-95
2	27.3 ± 0.1	24.5-29.2	75.4 ± 1.2	57.5-90.7	1/10/03-17/2/04	67.6 ± 16.8	32-139
3	27.4 ± 0.2	24.5-29.8	73.5 ± 1.0	57.5-90.7	20/10/03-22/3/04	77.6 ± 17.2	32-152
4	27.6 ± 0.2	25.0-31.2	72.5 ± 0.9	57.5-90.7	30/10/03-30/3/04	99.9 ± 19.3	39-163

Table 2. Cumulative effects of final instar larvae of *S. bipunctatus* and *Chrysopa* sp. on mortality of adult *D. brevipes*, corrected according to Abbott (1925).

Predator	Mortality %		
	Day 1	Day 2	Day 3
Chrysopa sp.	25.7	64.3	98.9
S. bipunctatus	21.3	60.8	95.8

Biology of Chrysopa *sp.*

The *Chrysopa* sp. studied here was found attacking *D. brevipes* in the wild, as well as other pseudococcid species, including *F. virgata*, *P. lilacinus* and *R. spinosus*. Lifespan of juvenile *Chrysopa* sp. averaged 20.2 (SE = 0.1) days at an average temperature of 29.5 °C and RH of 69%. The egg stage lasted 3 days, larvae fed for 8.5 days and the pupal stage lasted 8.7 days. Larvae underwent three moults, with the final stage lasting 2.8 (SE = 0.1) days. At an average temperature of 29.5 °C and 69% RH, *Chrysopa* sp. larvae consumed an average of 27.4 *D. brevipes* adults before pupating. Fourth instar larvae consumed the most, with an average of 18.8. Corrected prey mortality was nearly 100% by the third day (Table 2). Some *Chrysopa* sp. larvae attacked *D. brevipes* 2-3 times longer and 7-8 times wider than themselves. Female *Chrysopa* sp. laid an average of 53.8 eggs (range 33-110), with approximately 98.3% viable. Overall survival rate of *Chrysopa* sp. was 78.9% during the breeding trial period.

Discussion

Both predator species investigated in this study performed well under laboratory conditions and both have potential as augmentative biological control agents. They exhibited high levels of fecundity as well as short generation times (r-strategists), and their high levels of survivability in captivity (*S. bipunctatus* 82.5%, *Chrysopa* sp. 78.9%) suggests they might be successfully reared in large quantities. Other closely related *Chrysopa* and *Scymnus* spp. have been reared in captivity overseas, and have been successfully released as augmentative and classical biological control agents (Quayle 1941, Brader 1979, van Lenteren 1997, Flint and Dreistadt 1998, Vail *et al.* 2001).

Importantly, since *S. bipunctatus* and *Chrysopa* sp. attack other target species, such as *F. virgata*, they also may be effective at controlling these species or they may be bred on alternative hosts, including *F. virgata*, if *D. brevipes* numbers are small prior to the peak activity selected for optimal release times (or *vice versa*). For example, in Java *Scymnus* spp. are not normally numerous enough to control *P. citri* until the end of the dry season; however, they can be bred in large numbers during the rainy season on *P. lilacinus* and liberated against *P. citri* at the beginning of the dry season (DeBach and Hagen 1970). Variation in life history characteristics according to changes in ambient temperature and humidity, observed in both species, has been noted previously in biocontrol agents (*e.g.* Waterhouse 1993b), so captive breeding facilities can manipulate parameters such as diapause or eclosion times to capitalise on optimal release times. For example, diapausing adults of *Chrysoperla* (= *Chrysopa*) may be stored for up to 30 weeks at low temperatures (Tauber *et al.* 1993), and *S. bipunctatus* fecundity can be manipulated to optimize seasonal innoculations at crucial times during the life cycle of the pest (van Lenteren 2000).

These breeding trials are an essential first step for identifying natural candidates for augmentative biological pest control. Field trial release of these control agents is the next step in gauging their effectiveness *in situ*, and in determining the timing and optimal numbers to be released. It is also known that the attendant ants of *D. brevipes* (and other mealybugs) can act as a deterrent to potential biological control agents (González-Hernández *et al.* 1999, Williams and Watson 1990), so adequate measures to reduce or exclude ants from climbing trees infested with mealybugs need to be investigated. The findings in this study are encouraging, and it is hoped that mass rearing of *S. bipunctatus* and the *Chrysopa* species will provide a foundation for a biological pest management programme for the control of mealybug pests in Vietnam.

Acknowledgements

The two species of Lepidoptera were identified by Prof. Banpot Napompeth and Assoc. Prof. Kosol Charernsom from the National Biological Control Research Centre, Kasetsart University, Thailand. This project was supported in part by funding from the Ministry of Education and Training, Vietnam, and a Ford Motor Company Conservation and Environmental Grant to VTN.

References

ABBOTT, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265–267.

BIEN, PHAM VAN, TUYEN, BUI CACH and CHINH, NGUYEN MANH. 2000. *Handbook of pesticides*. Agricultural Publishing House, Ho Chi Minh City; 387 pp.

BRADER, L. 1979. Integrated pest control in the developing world. *Annual Review of Entomology* **24**: 225-254.

CAM, NGUYEN VAN. 1995. Status of biological control in Vietnam. Pp 103-115, in: P. Ferrar, Loke Wai Hong, A. Sivapragasam, A.A. Ismail, Lum Keng Yeang, Lim Guan Soon and J. Vos (eds), *Biological control as a cornerstone of integrated pest management for sustainable agriculture in Southeast Asia*. Proceedings of a workshop on biological control, Kuala Lumpur, Malaysia, 11-15 Sept. 1995; 221 pp.

DEBACH, P. and HAGEN, K.S. 1970. Manipulation of entomophagous species. Pp 429-458, in: P. DeBach and E.I. Schlinger (eds), *Biological control of insect pests and weeds*. Chapman and Hall Ltd., London; 844 pp.

ELMER, H.S. and BRAWNER, O.L. 1975. Control of brown soft scale in Central Valley. *Citrograph* **60**(11): 402-403.

FLINT, M.L. and DREISTADT, S.H.. 1998. *Natural enemies handbook. The illustrated guide to biological pest control*. University of California Press; 154 pp.

GONZÁLEZ-HERNÁNDEZ, H., REIMER, N.J. and JOHNSON, M.W. 1999. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* **44**: 47-58.

GOOLSBY, J.A., KIRK, A.A. and MEYERDIRK, D.E. 2002. Seasonal phenology and natural enemies of *Maconellicoccus hirsutus* in Australia. *Florida Entomologist* **85**: 494-498.

GULLAN, P.J. and CRANSTON, P.S. 2005. *The insects: an outline of entomology*. Third Edition. Blackwell Publishing, Malden, USA; 505 pp.

HODDLE, M.S. 2004. Restoring balance: using exotic species to control invasive exotic pests. *Conservation Biology* **18**: 38-49.

McGAVIN, G.C. 1993. *Bugs of the world*. Blandford, London; 192 pp.

PAUL, VAN MELE and HAI, TRAN VAN 1999. Pesticide use in orchards in the Mekong delta of Vietnam. Pp 529-533, in: A. Sivapragasam *et al.* (eds), *Fifth international conference on plant protection in the tropics: proceedings. Plant protection in the tropics: tropical plant protection in the information age*. Malaysian Plant Protection Society, Kuala Lumpur; 565 pp.

QUAYLE, H.J. 1941. *Insects of citrus and other subtropical fruits*, Comstock Publishing Company, Inc., Ithaca, New York; 583 pp.

TAUBER, M.J., TAUBER, C.A. and GARDESCU, S. 1993. Prolonged storage of *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* **22**: 843-848.

VAIL, P.V., COULSON, J.R., CAUFFMAN, W.C. and DIX, M.E. 2001. History of biological control programs in the United States Department of Agriculture. *American Entomologist* **47**: 24-49.

van LENTEREN, J.C. 1997. Benefits and risks of introducing exotic macro-biological control agents into Europe. *Bulletin OEPP/EPPO* **27**: 15-27.

van LENTEREN, J.C. 2000. Success in biological control of arthropods by augmentation of natural enemies. Pp 77-103, in: G. Gurr and S. Wratten (eds), *Biological control: measures of success*. Kluwer Academic Publishers, Dordrecht; 429 pp.

WATERHOUSE, D.F. 1993a. *The major arthropod pests and weeds of agriculture in Southeast Asia: distribution, importance and origin*. ACIAR Monograph No. 21, Canberra; 141 pp.

WATERHOUSE, D.F. 1993b. *Biological control: Pacific prospects - Supplement 2*. ACIAR Monograph No. 20, Canberra; 138 pp.

WATERHOUSE, D.F. and SANDS, D.P.A. 2001. *Classical biological control of arthropods in Australia*. CSIRO Entomology, Canberra; 559 pp.

WILLIAMS, D.J. and WATSON, G.W. 1990. *The scale insects of the tropical South Pacific Region. Part 3: The soft scales (Coccidae) and other families*. CAB International, Wallingford, UK; 267 pp.